Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

THE COMPLEMENT CONTENT OF ECK-FISTULA DOGS *

NOBLE P. SHERWOOD, CLARENCE SMITH, AND RAY WEST

From the Bacteriological and Physiological Laboratories of the University of Kansas, Lawrence

Many investigators have attempted to associate the liver with the production of complement.

Ehrlich and Morgenroth[1] observed a diminution of complement in dogs subjected to phosphorus poisoning with subsequent degeneration of the liver. Extirpation of the liver has been attempted by Nolf[2] and others, but in view of the almost insurmountable difficulties of the operation the work has been wholly unsatisfactory. Nolf next attempted to establish Eck's fistula in rabbits. Anastomosing the vena cava with the portal vein he obtained an immediate drop in complement, which lasted until the death of the animals 3 or 4 hours after the operation, and he concluded from this that the liver played an important rôle in the production of the complement. His work was later confirmed by Müller,[3] who also perfused serum through the liver and observed an increase in complement. Liefmann[4] was unable to confirm the results obtained by Müller. As a further check on the work he extirpated the liver from frogs, but observed no diminution of complement, the animals living, in some cases, 1 week.

More recently Dick[5] produced necrosis of the liver in dogs by means of chloroform poisoning, as suggested by Richards and Howland.[6] He observed a progressive drop in complement, lasting until the death of the animals some 48 hours afterwards. The complement content of normal dog serum was observed to vary from one-third to one-twelfth that of guinea-pig serum. The complement content of individual dogs showed a similar variation when the serum was titrated from time to time. In the animals suffering from chloroform poisoning, the complement content dropped to one-forty-fifth that of guinea-pig serum in 46 hours, just before the death of the animals.

Dick also produced destruction of liver cells by means of hydrazin sulfate. At the beginning of the experiment the complement content was one-fifth, after 48 hours it was one-tenth, while after 72 hours it was one-fortieth that of guinea-pig serum. The dog died a few hours later. Extensive necrosis of the liver was found microscopically. Dick concludes that complement is a proteolytic ferment which is either formed in the liver or is dependent on liver activity for its presence in the blood.

---

* Received for publication June 30, 1916.

[1] Gesammelte arbeiten zur Immunitätsforschung, 1904.

[2] Bull. de l'Acad. Roy. de Belg., 1908, Classe des Sc., p. 748. For a more complete bibliography of the work done on complement, see Zinser's Infection and Resistance, 1914, and also the article by Dick.[5]

[3] Centralbl. f. Bakteriol., I, O., 1911, 57, p. 577.

[4] Weicharts Jahresbericht, 1912.

[5] Jour. Infect. Dis., 1913, 12, p. 111.

[6] Jour. Exper. Med., 1909, 11, p. 344.

It will be observed that in all the experiments cited, the animals died within a few hours or at most a few days after the work was started. In the work of Nolf and others on Eck's fistula only 3 or 4 hours elapsed before the animals succumbed, while Dick's chloroformed animals lived only about 48 hours.

In view of the fact that dogs in which Eck's fistula hal been established by Dr. S. A. Matthews and one of us of the department of physiology, were surviving in good health almost indefinitely, it was thought worth while to study the complement content of these animals and to compare it with the progressive and noticeable atrophy, fatty degeneration and infiltration, and necrosis of the liver which followed the operation. For this work those dogs were selected which had no normal antihuman or antisheep hemolysins in their sera.

Blood was obtained from the external saphenous vein from one to several times preceding the operation and the complement content determined and compared with that of guinea-pig serum. Two hundredths cubic centimeter, that is, 0.2 c.c. of a 1 : 10 dilution, of guinea-pig complement was adopted as a unit for comparison.

The estimation of complement was carried out as follows:

At first both antihuman and antisheep hemolytic amboceptors from rabbits immunized against the respective cells were employed. These were titrated to determine the unit of each. In the case of the antihuman hemolytic amboceptor, we used the method of titration recommended by Noguchi[7] and others. Each hemolytic test tube contained 0.1 c.c. of a 10% suspension of washed human red-blood corpuscles, 0.1 c.c. of its respective dilution of amboceptor, 0.5 c.c. of a 1 : 10 dilution of active guinea-pig complement, and enough physiologic salt solution to make the final volume 1 c.c. These tubes were incubated in a water bath at 37.5 C. for 30 minutes. It was found that the tube containing 0.1 c.c. of 1 : 120 dilution of amboceptor held the highest dilution showing complete hemolysis. This was considered the titer of the serum. In titrating dog complement, each hemolytic tube contained 0.1 c.c. of a 10% suspension of washed human red-blood corpuscles, 1 unit of amboceptor, its respective amount of dog serum, and physiologic salt solution to make the volume 1 c.c. These tubes were then incubated in a water bath at 37.5 C. for 30 minutes and examined. They were next placed in the ice box over night and in the morning read again. The least amount of dog serum bringing about complete hemolysis was considered the titer of the dog complement.

In titrating dog serum varying amounts of a 1 : 10 dilution, as well as varying amounts of straight serum, were used. Inasmuch as 0.02 c.c. of guinea-pig complement was the unit for comparison, if it was found that 0.1 c.c. of straight dog serum was the titer of the dog complement, then the latter would be one-fifth as strong as guinea-pig complement.

In working with antisheep amboceptor, 0.5 c.c. of a 1 : 1000 dilution was found to be a unit; that is, it produced complete hemolysis in a tube containing 0.5 c.c. of a 5% suspension of washed sheep corpuscles, an excess of complement, and sufficient salt solution to make the final volume 2.5 c.c. Complement was then titrated against this unit of amboceptor.

---

[7] Serum Diagnosis of Syphilis and the Butyric Acid Test for Syphilis, 1910.

In most of the work the antihuman hemolytic amboceptor only was used, as it was very satisfactory.

The titer of the dog complement was then determined for several days preceding and immediately before and after the operation. It was then titrated 2½, 6, 16, and 24 hours after the operation and at frequent intervals thereafter until the dogs were killed and the liver removed for microscopical examination. Animals were killed and tissue was obtained for study 30, 60, and 90 days after Eck's fistula had been established. Liver tissue from apparently normal dogs was obtained for comparison.

Table 1 records the results of the titrations. The data on Dogs 7 and 8 are also shown in the form of curves in the chart.

TABLE 1

THE COMPLEMENT TITER IN ECK FISTULA DOGS

Dog	Titer of Dog Complement in Terms of Guinea-Pig Complement									Anesthetic Used	Remarks
	Immediately Before Operation	Immediately After Operation	2½ Hours After Operation	6 Hours After Operation	16 Hours After Operation	24 Hours After Operation	30 Days After Operation	60 Days After Operation	90 Days After Operation		
1	1/8	....	....	....	....	1/5	1/5	....	....	Ether	Posted 30 days
2	1/5	....	....	....	....	1/5	1/8	....	....	Ether	Posted 30 days
3	1/10	....	....	....	....	1/12	....	....	....	Ether	Killed by mistake
4	1/5	1/10	1/10	1/8	1/5	1/5	1/5	1/5	....	Ether	Posted 60 days
5	1/10	1/15	1/12	1/10	1/10	1/8	1/5	1/8	1/5	Ether	Posted 90 days
6	1/6	1/12	1/10	1/8	1/5	1/5	1/5	1/8	....	Ether	Still living
7	1/5	1/10	1/8	1/8	1/5	1/5	1/5	1/8	....	Ether	Still living
8	1/8	1/30	1/15	1/5	1/8	1/5	1/5	....	....	Chloroform	Died from ammonia poisoning
9	1/4	1/25	1/15	1/5	1/5	1/5	1/5	1/10	....	Chloroform	Still living

The titer of the complement of individual normal dogs has been observed to fluctuate from one-fourth to one-twelfth that of guinea-pig complement.

Microscopically the livers of the dogs examined at 30, 60, and 90 days after the establishment of Eck's fistula, disclosed a progressive fatty degeneration and infiltration with necrosis of the liver cells. There was no corresponding decrease in complement accompanying the degenerative processes in the liver. Whether there will occur a drop in complement when the liver has completely degenerated, remains to be determined. Dogs are being carried over until next year for that purpose.

Ether was used as an anesthetic in 7 of the operations. In the case of the first 3 dogs, data on the complement unfortunately were not obtained until 24 hours after the operation when it was back to normal. In the next 4 dogs, immediately following the operation there was a drop in complement of about one-half, the complement returning to normal within 16 hours. In the 2 dogs operated upon under chloroform anesthesia the drop was very much greater, the titer going down to approximately one-fourth to one-sixth of the titer before the operation and returning to normal within 6 hours after the operation.

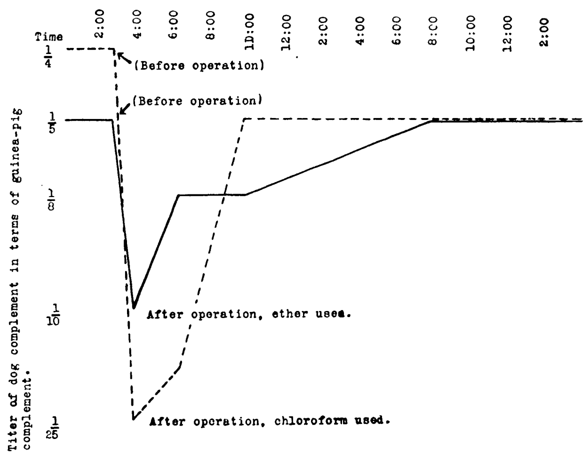

Chart 1. The broken line shows the titer of complement before and after the establishment of Eck's fistula when chloroform was used as the anesthetic. The solid line shows the titer of complement before and after the establishment of Eck's fistula when ether was used as the anesthetic.

We have here several factors all or part of which may contribute to the temporary drop in complement: (1) the interference with the normal function of the liver, (2) the sudden shunting of the portal circulation into the general circulation, (3) the effect of the anesthetic on the various tissue cells, leading not only to disturbed function but to change of blood constituents, (4) the presence of the anesthetic in the circulating blood in contact with the complement, and (5) the shock of the surgical operation. In view of the fact that the drop in complement was much less under ether than it was under chloroform anesthesia, it would appear that the 3rd and 4th factors may be largely responsible for the immediate fall in complement.

In their work on Eck-fistula dogs, Matthews and Miller[8] emphasized the danger of the formation of adhesions following the operation, and the establishment of a collateral circulation for the liver. In the work here recorded great care was exercised to avoid this possibility. Adhesions were looked for carefully post mortem and data on dogs showing adhesions and collateral circulation were not included in the report. A few weeks after the operations the feces of the dogs became grayish-white in color, indicating the absence of bile. Symptoms of ammonia poisoning, as pointed out by Hahn, Massen, Nenchi, and Pawlow,[9] and Matthews and Miller,[8] could be easily induced by increasing the amount of meat in the diet. These were fair indications that a collateral circulation had not been established. In a few cases hemolytic substances appeared in the blood, but these could be eliminated easily by putting the dogs on a bread and water or milk diet for a couple of days. The complement was usually at a low normal, that is, one-eighth to one-twelfth that of guinea-pig serum, but normal dogs showed a corresponding drop in complement when put on the same diet.

In regard to tissue changes in the liver following the Eck-fistula operation, Matthews and Miller make the following statement: "It is well known that after an Eck's fistula the liver soon begins to undergo fatty necrosis, which eventually invades the whole organ. This change in the nutrition of the organ may be responsible, in large measure, for the changes in metabolism so noticeable after an Eck's fistula." Macroscopically the liver showed a progressive atrophy. The rate of degeneration varied with different dogs, but was usually almost at a maximum in about 90 days. However, Dog 5 had not reached that stage at 90 days. In Dog 9, which at the writing of this report had been carried along 70 days, the liver was not palpable. The complement content on the 70th day was one-tenth that of guinea-pig serum. This dog, as well as normal dogs, had been kept off meat and fed on table scraps for 4 days previous to the titration. The sera of the normal dogs showed a complement content of one-tenth to one-twelfth that of guinea-pig serum at this time; previous to the change of diet it had been one-fifth.

CONCLUSIONS

The complement content of normal dog serum may vary in the same animal from one-fourth to one-twelfth that of guinea-pig serum.

---

[9] Arch. f. Exper. Path. u. Pharm. 1893, 32, p. 161.

[8] Jour. Biol. Chem., 1913, 15, p. 87.

Immediately following the establishment of Eck's fistula there is a temporary drop in complement lasting not more than 16 hours as a rule.

There is a much greater drop in complement under chloroform anesthesia than under ether anesthesia. The return to normal is apparently just as rapid when only sufficient chloroform is used to produce the surgical anesthesia.

The complement content of dog serum following the establishment of Eck's fistula was normal within from 6 to 16 hours after the operation and remained normal for at least 90 days (last observation) in the dogs studied.

There is no corresponding drop in complement paralleling or accompanying the degeneration of the liver.

There does not seem to be sufficient evidence, as yet, to warrant the assumption that the liver plays a more important rôle in the production of complement than do other organs or tissues of the body.